Folding and Misfolding of Designed Heteropolymer Chains with Mutations.

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Abstract

We study the impact of mutations (changes in amino acid sequence) on the thermodynamics of simple protein-like heteropolymers consisting of N monomers, representing the amino acid sequence. The sequence is designed to fold into its native conformation on a cubic lattice. It is found that quite a large fraction, between one half and one third of the substitutions, which we call 'cold errors', make important contributions to the dynamics of the folding process, increasing folding times typically by a factor of two, the altered chain still folding into the native structure. Few mutations ('hot errors'), have quite dramatic effects, leading to protein misfolding. Our analysis reveals that mutations affect primarily the energetics of the native conformation and to a much lesser extent the ensemble of unfolded conformations, corroborating the utility of the "energy gap" concept for the analysis of folding properties of protein-like heteropolymers.

The use of genetically altered proteins has become an important experimental tool for studying the physical principles that determine the thermodynamical stability and folding mechanisms of proteins [1]. Many experiments suggest that the effect of mutations, in most cases is specific to the location and identity of the mutated amino acid, consistent with the view that mutations most strongly affect the properties of the native structure. This conclusion was made also in the theoretical analysis of the statistical mechanics of heteropolymers representing simple physical models of protein. It was argued in [2,3] that the basic properties of the denatured state ensemble are self-averaging, i.e. they depend on amino acid composition rather than on specific sequences. In contrast, the energetics of the native state are very sensitive to specific details of a sequence. An alternative view was suggested that mutations may affect rather the denatured state, if the latter has elements of structure [4]. Of central importance in this quest is the identification of the role individual amino acid residues play in the cooperative phenomenon of folding (cf. e.g. Ref. [5] and refs. therein).

A useful theoretical approach to study protein folding is a simplified lattice model. Such models have proved helpful, not only for elucidating generic features of folding proteins [6], but also for the evaluation of specific nucleation sites for folding kinetics [7,8]. The goal of this Letter is to elucidate, for the same model, the impact of mutations on protein *thermodynamic stability*.

To this end, the thermodynamics of protein-like heteropolymer chains consisting of N monomers, representing the amino acid sequence, is studied on a cubic lattice for modeling folding of proteins to their native structure. The sequence is designed to fold into the native conformation, shown in Fig. 1; this conformation and the sequence are identical to that previously studied in [7,10]. Mutations in the designed amino acid sequence are introduced by replacing a single monomer with a different one.

The configurational energy of the chain is given by

$$E = \frac{1}{2} \sum_{i,j}^{N} U_{m(i),m(j)} \ \Delta(|\mathbf{r}_i - \mathbf{r}_j|), \tag{1}$$

where $U_{m(i),m(j)}$ is the effective interaction potential between monomers $m(i)$ and $m(j)$, $\mathbf{r}_i$ and $\mathbf{r}_j$ denote their lattice positions and $\Delta(x)$ is the contact function. In Eq. (1), the pairwise interaction is different from zero when i and j occupy nearest-neighbor sites, i.e. $\Delta(1) = 1$ and $\Delta(a) = 0$ for $a > 1$. In addition to these interactions, it is assumed that on-site repulsive forces prevent two amino acids to occupy the same site simultaneously, so that $\Delta(0) = \infty$.

In what follows we shall consider a twenty-letter representation of protein sequences where U is a 20×20 matrix. This matrix is taken from the work [9] (Table VI) where it was derived from frequencies of contacts between different aminoacids in protein structures. We study, by means of Monte-Carlo (MC) simulations, the dynamics of a 36-monomer chain characterized by a polymer sequence, denoted as S_{36}, designed by minimizing the energy in the target (native) conformation [10] (see Fig. 1). In the units we are considering, the energy of S_{36} in its native structure is $E_{nat} = -16.5$, and the folding transition temperature $T_f = 0.40$. These values correspond to the normalized values $\tilde{E}_{nat} = -58.20$ and $\tilde{T}_f = 1$ obtained for this sequence in Ref. [10]. MC simulations of folding are performed using a standard algorithm described extensively in the literature [11], in which, at each MC step, a monomer is picked up at random and corner flips and crankshaft moves are considered.

Mutations in the designed sequence are introduced by replacing a single monomer in S_{36} by a monomer of a different type. In our case, there exist 19 such possible substitutions for each monomer in S_{36}, implying $36 \times 19 = 684$ different sequences to study. Actually, a systematic behavior of folding can be deduced by analyzing a relatively small number of such altered chains. To show this, we started our analysis by choosing a monomer of S_{36} arbitrarily, say the 17-th monomer, and studied the folding dynamics of the corresponding 19 altered chains. The MC simulations were performed up to a maximum number of $15 \cdot 10^6$ steps, for the temperature $T = 0.28$ and averaging over 12 different starting random coil configurations for each altered sequence. By repeating the same procedure for other few selected sites along the original chain, it is possible to conclude that the behavior of altered chains can be generally classified into three categories:

(1) chains which still fold to the native structure,

(2) chains which fold to a unique compact structure, but different than the native one, and

(3) chains which, although becoming compact, do not fold to a unique structure at all during simulations.

To characterize quantitatively the above three different behaviors, we find that the quantity ΔE_{loc} defined as the difference between the energies of the altered and the intact ("wild-type") chain, both calculated in the native configuration, plays a key role. More precisely, such local energy difference, $\Delta E_{loc}[m'(i) \to m(i)]$, for a mutation at site i, where the monomer $m(i)$ in S_{36} is replaced by a monomer $m'(i) \neq m(i)$ is given by

$$\Delta E_{loc}[m'(i) \to m(i)] = \sum_{j \neq i} \left(U_{m'(i),m(j)} - U_{m(i),m(j)}\right) \Delta(|\mathbf{r}_i - \mathbf{r}_j|). \tag{2}$$

We have calculated all the 684 values of $\Delta E_{loc}[m' \to m]$, and found they fall in the range $0 \leq \Delta E_{loc}[m' \to m] < 5.66$. We classify the impact of mutation by the ability of the mutated sequence to fold into or close to the native conformation. We define the degree of folding Q similar to earlier publications (see e.g. [12]) as the fraction of correctly formed contacts ($Q = 1$ corresponds to the native state and $Q \ll 1$ corresponds to misfolded states).

The following rules are obtained from our results:

(1) $\Delta E_{loc}[m' \to m] < 1$: the altered chain always folds to the native structure ($Q = 1$).

(2) $1 < \Delta E_{loc}[m' \to m] < 2.5$: the altered chain folds to a unique structure, sometimes different than the native one, with Q being smaller but close to one. In some cases, however, folding to the native structure may still occur ($Q = 1$).

(3) $2.5 < \Delta E_{loc}[m' \to m] < 4$: twilight zone: for some mutations, chains fold into near native structure with $Q \sim 1$, other mutations lead to misfolding with $Q \ll 1$.

(4) $\Delta E_{loc}[m' \to m] > 4$: the altered chain does not fold to a unique structure at all during the simulation time, and now $Q \ll 1$.

For a given site i, we can therefore classify the 19 possible mutations according to the rules (1) and (3,4). For rule (2), additional information about the dynamical behavior of the

chain is required. We find that 73.675% mutations fall into the first class $\Delta E_{loc}[m' \to m] < 1$, 26.3% into the second class $1 < \Delta E_{loc}[m' \to m] < 4$, and the rest 0.015% into the class (4). Thus, a relatively large fraction of mutations yield altered chains still folding into the native structure, some mutations lead to limited misfolding ($Q \approx 1$) and only a small fraction of mutations leads to complete misfolding.

Mutations at a given site may yield values $\Delta E_{loc}[m' \to m]$ which do not correspond to a single class, i.e. sometimes values smaller and larger than one occur at the same site. However, assuming that mutations are not selective, i.e. that they all occur with equal probability at a single site, an approximate scheme can be envisaged to classify the different sites according to the average magnitude of the damage caused by mutations. This is done by calculating the average value of $\Delta E_{loc}[m' \to m]$ for each site i as,

$$\Delta \bar{E}_{loc}(i) = \frac{1}{19} \sum_{m'} \Delta E_{loc}[m'(i) \to m(i)]. \tag{3}$$

In this way, to each site i is associated a mean value $\Delta \bar{E}_{loc}(i)$ and the following simple scheme emerges:

(1) When $\Delta \bar{E}_{loc}(i) < 1$, chains having mutations at site i are likely to belong to the first category, i.e. on average they fold to the native structure and we denote i as a 'cold' site,

(2) when $1 < \Delta \bar{E}_{loc}(i) < 2$ they behave on average as in the second category mentioned above, and i is denoted as a 'warm' site. Finally,

(3) when $\Delta \bar{E}_{loc}(i) > 2$ the resulting altered chains are likely to yield unfolded structures, and the site is denoted as a 'hot' site.

We have classified the 36 monomers of S_{36} according to this scheme as shown schematically in Fig. 1. We find that 27 sites can be considered as cold sites, 6 as warm sites and only 3 as hot ones (see Table I). Thus, about 75% of the heteropolymer chain admits single error substitutions in the correct amino acid sequence yielding altered chains still folding to the native structure. Only in a relatively small fraction of the chain (about 10%), mutations have catastrophic effects leading to complete misfolding. Additional simulations have confirmed the general trend predicted by this empirical scheme.

In order to gain deeper insight into the observed behavior of chains with mutations, we evaluated the density of states $\nu(Q, E)$ which is the logarithm of the number of conformations having given values of the degree of folding Q and of the energy E, using histogram technique [13]. To this end long runs ($\approx 30 \cdot 10^6$) were performed for the "wild-type" sequence and mutations S17L ($\Delta E_{loc} = 1.12$), P16D ($\Delta E_{loc} = 1.44$), D6E ($\Delta E_{loc} = 0.44$) and W16D ($\Delta E_{loc} = 2.30$). In the latter case the chain folded to a conformation, different from the native one, having $Q = 0.85$. (We used the following notation for mutations: S17L means that at site 17 amino acid of type "S" is replaced by amino acid of type "L").

Frequencies of appearance of conformations with different values of Q and E were evaluated, and the analysis described in [14] was applied to derive $\nu(Q, E)$. Fig. 2 shows the 3D plots for the density of states for the "wild-type" sequences as well as S17L for mutation. The remaining plots are qualitatively similar to the one corresponding to mutation S17L and are not shown here to save space. The pronounced boundary of "continuous" spectrum at $E_c \approx -14$ can be seen on all these plots. This is the lowest limiting energy which non-native conformations (with Q in the range $0, ..., 0.5$) have. Comparing Fig. 2a with Fig. 2b,2c one can see that mutations do not affect E_c, i.e. the spectrum of energies of non-native conformations. In contrary, the most pronounced differences between the original sequence and mutants can be seen in the vicinity of the rightmost of the low-energy "tail" corresponding to native and near-native conformations. One can define the physically meaningful "energy gap" as the difference in energy between the native state and lowest energy *misfolded* (low Q) conformations i.e. $E_{nat} - E_c$. Fig. 2 also helps to understand the difference between the definition of the energy gap used in [14,15] and the one used in a more recent work [16]. Klimov and Thirumalai define "energy gap" as the difference between the energy of the native state conformation and nearest to it in energy. It can be seen from Fig. 2 that the definition used in [16] concerns conformations nearest to the native in the high-Q "tail", i.e. differing from the native state by a monomer flip on the edge of the structure.

Now, the results of our analysis can be easily rationalized. The energy gap for the original sequence is -2.5 (i.e. $8k_BT$ at the temperature of simulations). Mutations eliminating the

energy gap (having $\Delta E_{loc} > 2.5$) are in most cases disruptive, so that mutant sequences lose all useful features of design, behaving effectively as random ones, which, with some low probability, can still fold [15]. Mutations with less pronounced energetic consequences preserve the "high-Q" tail in histograms in Fig. 2 (sometimes a new conformation from this tail may become native) and sequences are still able to fold. These findings once again emphasize the role of properly defined "energy gap" as an important factor determining folding properties.

The amino acid design tends to place strongly interacting amino acids in internal positions which form most contacts, creating therefore a network of interactions inside the molecule which carries significant part (up to 30%) of the total stabilization energy for the native structure. As a consequence protein structure is most sensitive to mutations in these positions. Moreover, as was argued in Ref. [8], internal stabilizing amino acids are most likely to form their correct contacts in the transition state, thus these residues can serve as nucleation ones. For this reason, residues identified in [7] as nucleation sites are also "hot" positions as found in the present study where mutations have most pronounced impact on stability.

Our results provide additional insights into the important issue of what part of the energy landscape of a protein is most affected by mutations. We showed that the native and near-native conformations are most affected while the impact of mutations on the energies of the ensemble of misfolded states are relatively less pronounced. While this result differs from previous assertions [4] we believe that it makes clear physical sense. Indeed, in optimized sequences, each amino acid forms mostly favorable contacts in the native state. Correspondingly, mutations are doomed to replace many such interactions by less favorable ones, significantly affecting the energy of the native state. In contrast, in the unfolded state which represents an ensemble of conformations, each amino acid forms flickering contacts with many other amino acids and the impact of mutations on the denatured state "averages out" over a multitude of conformations of the denatured state ensemble. The assertion [4] that mutations affect rather the denatured state was based on the analysis of very short (16

monomers) 2-dimensional chains, and the small size and limited conformational freedom in two dimensions, is likely to have hindered the effect of averaging over the conformational ensemble of the denatured state which we observe in longer 3-dimensional chains.

An important issue is how general our results are, in particular how do mutations affect folding of longer chains. The energy gap (correctly defined, see Fig.2 and accompanying discussion) must be extensive in chain length in order ensure fast folding to stable native conformation [18,3]. To this end longer chains should be more tolerant to point mutations since they cause smaller relative change of energy gap. However, still mutations at different positions will have different impact, and this factor may be crucial for longer chains as well especially taking into account their multidomain behavior. In that case mutations will affect folding of a domain to which mutated aminoacid belongs leaving stability of other domains unchanged [17].

Our study suggests that protein structures may be remarkably tolerant to many mutations in a number of 'cold' positions. Experimental studies [5] indicate that this is indeed the case. It is likely that gradual changes may accumulate to provide new proteins with different structure and function. We believe that such evolutionary phenomenon can be observed and studied in the realm of a simple folding model. It is the matter of future work justify or rule out this optimistic assertion.

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TABLES

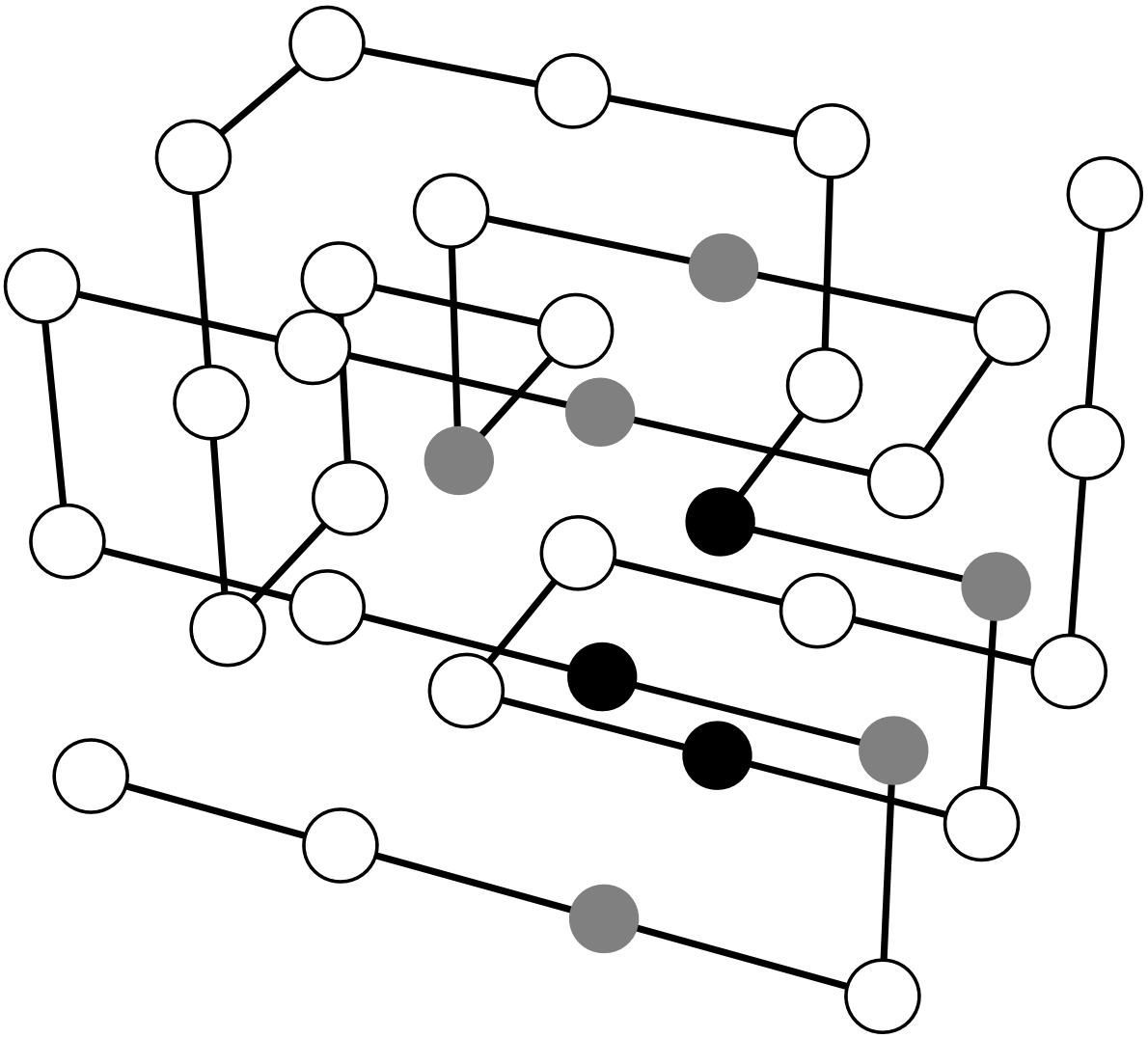
TABLE I. The average values of $\Delta\bar{E}_{loc}$ for each site of S_{36}. Bold numbers indicate the hot sites.

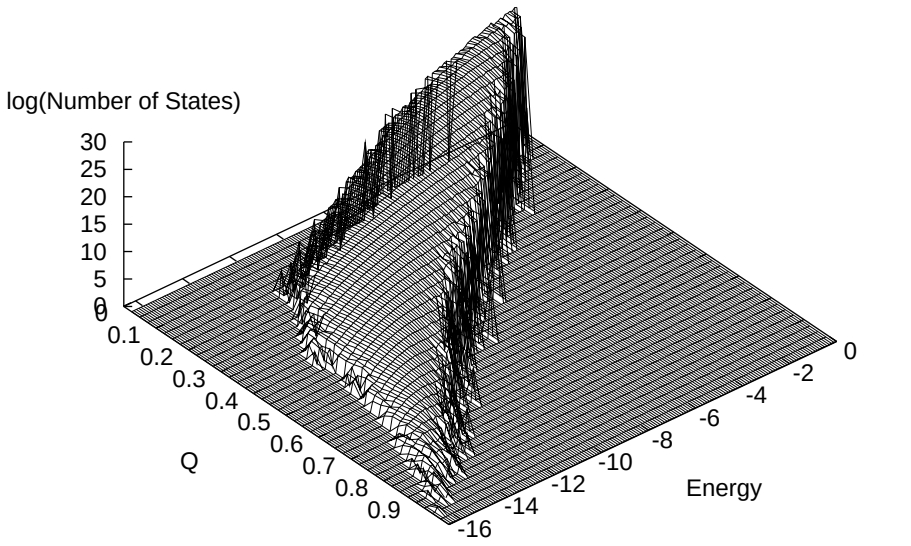
site	$\Delta\bar{E}_{loc}$	site	$\Delta\bar{E}_{loc}$	site	$\Delta\bar{E}_{loc}$	site	$\Delta\bar{E}_{loc}$
1	0.24	10	0.64	19	0.38	28	1.31
2	0.73	11	1.46	20	0.63	29	0.95
3	1.94	12	0.50	21	0.65	30	**2.48**
4	0.75	13	0.55	22	0.40	31	0.99
5	1.09	14	1.85	23	0.28	32	0.78
6	**2.79**	15	0.88	24	0.77	33	0.68
7	0.77	16	1.79	25	0.44	34	0.42
8	0.27	17	0.80	26	0.54	35	0.66
9	0.09	18	0.38	27	**3.46**	36	0.26

FIGURES

FIG. 1. The native structure and sequence S_{36} of the model 36-mer. Schematic representation of the three types of sites (different gray scale) characterizing single mutations in the designed amino acid sequence according to the value $\Delta\bar{E}_{loc}(i)$ (see text). With most mutations occuring at a cold site (white balls), the chain can still fold to the native structure but the average folding time is considerably longer than for the designed sequence. For most mutations occurring at a warm site (grey balls) the chain folds to a unique structure, close to but different than the native one. For most mutations occurring at the hot sites (dark balls), the chain does not fold to a unique structure, during simulation time and different states of comparable local energy minima are reached.

FIG. 2. The density of states $\nu(Q, E)$ for: (a) "wild-type" sequence, (b) mutation S17L

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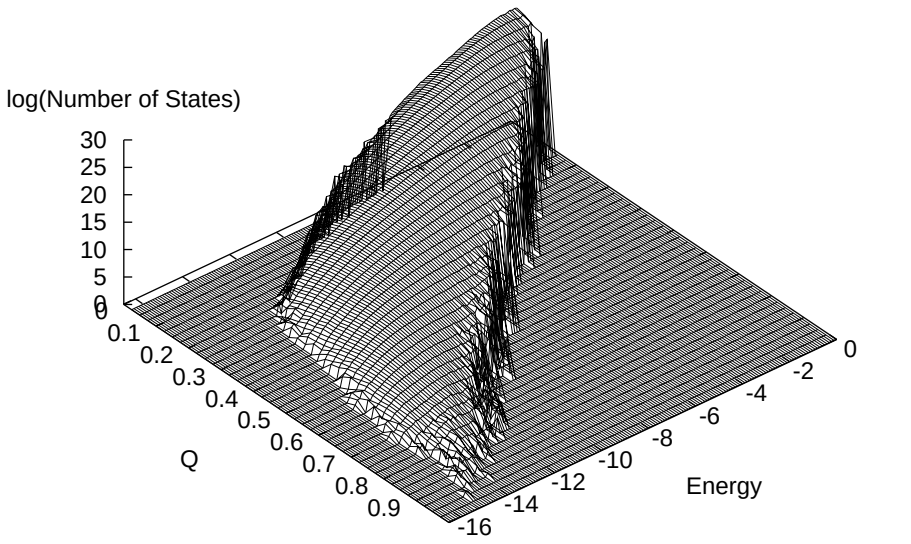
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Energy